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lecular ecology studies of species radiations: current research gaps, portunities, and challenges

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Abstract

Understanding the drivers and limits of species radiations is a crucial goal of evolutionary genetics and molecular ecology, yet research on this topic has been hampered by the notorious difficulty of connecting micro- and macro-evolutionary approaches to studying the drivers of diversification. To chart the current research gaps, opportunities, and challenges of molecular ecology approaches to studying radiations, we examine the literature in the journal MOLECULAR ECOLOGY and re-visit recent high-profile examples of evolutionary genomic research on radiations. We find that available studies of radiations are highly unevenly distributed among taxa, with many ecologically important and species-rich organismal groups remaining severely understudied, including arthropods, plants, and fungi. Most studies employed molecular methods suitable over either short or long evolutionary time scales, such as microsatellites or Restriction site Associated DNA sequencing (RAD-seq) in the former case and conventional amplicon sequencing of organellar DNA in the latter. The potential of molecular ecology studies to address and resolve patterns and processes around the species level in radiating groups of taxa is currently limited primarily by sample size and a dearth of information on radiating nuclear genomes as opposed to organellar ones. Based on our literature survey and personal experience, we suggest possible ways forward in the coming years. We touch on the potential and current limitations of whole genome sequencing (WGS) in studies of radiations. We suggest that WGS and targeted ('capture') resequencing emerge as the methods of choice for scaling up the sampling of populations, species, and genomes, including currently understudied organismal groups and the genes or regulatory elements expected to matter most to species radiations.

Introduction

Understanding the origin and maintenance of biological diversity has long been the 'holy grail' of molecular ecology and evolutionary biology. Many aspiring biologists starting to deal with these important topics will find that the literature is largely divided into two rather contrasting worlds of approaches championed by different communities of researchers. On the one hand, macro-evolutionary approaches based on the tools of phylogenetics and comparative biology have been extremely helpful for gaining a retrospective understanding of spatial patterns and ecological correlates of species diversification over long time scales (Barraclough & Vogler 2000; Linder 2008; Cavender-Bares *et al.* 2009; Kozak & Wiens 2010). On the other hand, micro-evolutionary approaches based on the analytical tools of population genetics have proven to be highly useful for understanding the actual mechanisms acting during population divergence and speciation 'in real-time', as exemplified by rapid recent progress in the field of speciation genomics (Smadja & Butlin 2011; The rie Curie SPECIATION network 2011; Feder *et al.* 2012; Nosil 2012; Seehausen *et al.* 2014). Only recently, connecting these two conceptual worlds within one single convincing study or research program has come truly within reach for many groups of taxa, thanks mainly to the availability of high-throughput genomic technologies and analytical tools for analyzing these novel types of data across the relevant taxonomic, spatial, temporal, and hierarchical / organismal scales (The Heliconius Genome Consortium 2012; Brawand *et al.* 14; Lamichhaney *et al.* 2015; Charlesworth & Charlesworth 2016; Novikova *et al.* 2016).

The wide-spread disconnection between research at the micro- and macro-scales of evolution in most organismal groups is particularly problematic in the context of evolutionary radiations, that is, bursts of speciation typically associated with unusually high diversification rates (Rundell & Price 2009), or phrased differently, the "dramatic proliferation of taxa in a clade" (Simões *et al.* 2016). Since radiations (regardless whether initiated through adaptive or non-adaptive causes) are the source of most biodiversity on Earth (Schluter 2000; Rundell & Price 2009), understanding the molecular and population genetic mechanisms that drive or

constrain radiations is no doubt an extremely worthwhile goal. A particularly hotly debated process in this context is adaptive radiation, i.e. radiation resulting from natural selection ving divergence of an ancestral species into daughter species that are better able to exploit open niches (Glor 2010), often in the face of gene flow from their progenitors (Schluter 2000; Gavrilets *et al.* 2005). It is clear that the outcome of this enigmatic process depend strongly on the interplay of the "big four" in population genetics: mutation, selection, migration (gene flow), and drift. Yet, most currently available knowledge of radiations (including adaptive radiations) stems from macro-evolutionary studies (Barraclough & Vogler 2000; Linder 2008; Jablonski 2008; Cavender-Bares *et al.* 2009; Glor 2010; Kozak & Wiens 2010), which tend to be correlative by nature and thus limited in ntifying the precise molecular and population genetic mechanisms responsible for the diversification process. Molecular ecology approaches based on natural genetic variation present within and between diverging populations – based firmly on the concepts of population genetics – hold great promise for tackling this task and identifying the mechanisms and drivers of species radiations. This becomes clear from a rich body of conceptual literature (e.g. Seehausen 2004; Gavrilets & Vose 2005; Givnish 2010; Abbott *et al.* 2013; Arnold 2016; Charlesworth & Charlesworth 2016) including studies of the so-called ´speciation continuum´ (Feder *et al.* 2012; Nosil 2012), and from inspecting the rapidly growing toolbox of population genomics (Nielsen 2005; Ellegren 2014).

The authors of the present paper form part of a trans-disciplinary research project aimed at understanding the drivers and limits of species radiations using the tools of ecology, phylogenetics, and population genetics. While developing and implementing this research, we noticed the relative feasibility of integrating ecology and phylogenetics for dressing macro-evolutionary patterns of radiations (notwithstanding potential issues with violating simplifying assumptions when applying ecological principles to comparative methods; Emerson & Gillespie 2008). The well developed framework for integrating ecology d phylogenetics stands in contrast with the perceived practical and conceptual challenges of firmly integrating population genetics into this research to pinpoint the mechanisms,

drivers, and constraints of the evolutionary process. In our case, the challenges posed by population genetics were manifold and ranged from practical issues such as the need to sample sufficient numbers of individuals from many populations and species, often under difficult field conditions (e.g. neotropical rain forests), to selecting efficient genomic and analytical tools for capturing changes in allelic frequencies for many populations and species, including those genome regions expected to matter most to the evolutionary process. Numerous interactions at recent scientific meetings and conferences suggest to us that many / most researchers and students of species radiations are confronted with similar challenges, and even recent high-profile studies of classical 'model radiations' are clearly

ected by trade-offs between sampling populations, species, and genomes (e.g. The

liconius Genome Consortium 2012; Brawand *et al.* 2014; Lamichhaney *et al.* 2015; Novikova *et al.* 2016). We were thus motivated to explore recent progress in molecular ecology and population genetic approaches to studying species radiations in one of the leading subject journals, MOLECULAR ECOLOGY. Our goal was to sketch current research gaps in molecular ecology studies of radiations and to come up with practical suggestions for future research. Our hope was to help enable aspiring researchers to successfully negotiate the trade-offs arising from sampling large numbers of populations, species, and genomes in radiating taxa.

To facilitate the present contribution, we first examined the literature on species radiations in MOLECULAR ECOLOGY covering a 20-year period, using a stringent set of search terms, with the goal of yielding a manageable core set of articles employing molecular ecology approaches for studying radiations. We then cross-tabulated a range of biological

d technical attributes of the reviewed studies. We placed particular emphasis on the geographic and taxonomic distribution of available studies and the different types of molecular genetic tools used. We also examined the effects of sampling different numbers of individuals, populations, and species from radiating clades and different numbers of polymorphism from their genomes. We used the results to sketch current research gaps, opportunities, and challenges in molecular ecology studies of radiations. We did not place

emphasis on theoretical questions at the interface of genotype, phenotype, and fitness during species diversification, as these issues have been dealt with elsewhere (e.g. Salamin *et al.* 2010; Smadja & Butlin 2011; The Marie Curie SPECIATION network 2011; Simões *et al.* 2016; Kostikova *et al.* 2016). We close by suggesting priorities for future research, with special emphasis on the exciting opportunities provided by novel tools now available to molecular ecologists around the world.

Molecular ecology studies of radiations – the last 20 years

We carried out a targeted review of studies on species radiations published in MOLECULAR ECOLOGY by accessing Web of Science in December 2015, using the search terms 'radiation', 'speciation' and 'population'. We double-checked with Google Scholar that no relevant studies had been overlooked. After filtering the results to include primary literature only (excluding review and perspective articles), our search yielded 93 studies published between 1996 and 2015 (**Table 1; Table S1;** all listed in **reference section** of this paper). Our time points for accessing the literature and for analyzing and summarizing the results depended on the work flow of ongoing research projects in our labs; particularly relevant studies published more recently are discussed here as well, whenever appropriate.

The reviewed studies include numerous noteworthy contributions that are likely to impact on future molecular ecology studies of radiations. They address some of the most hotly debated evolutionary radiations in animals and plants, including cichlids (e.g. Schliewen *et al.* 2001; Albertson *et al.* 2014; Colombo *et al.* 2013; Keller *et al.* 2013; Manousaki *et al.* 2013; Ford *et al.* 2015), sticklebacks (Gow *et al.* 2006; Berner *et al.* 2010; Deagle *et al.* 2013; Feulner *et al.* 2013), *Anolis* lizzards (Glor *et al.* 2005; Ng & Glor 2011; Munoz *et al.* 2013), *Heliconius* butterflies (Nadeau *et al.* 2013), Darwin´s finches (Petren *et al.* 2005), plants of the Hawaiian silversword alliance (Lawton-Rauh *et al.* 2007), oak trees (Cavender-Bares *et al.* 2015), and bromeliads (Palma-Silva *et al.* 2011). Due to the targeted nature of our search and the specific search terms used, our literature review is certainly not exhaustive. Nevertheless, it should be broadly representative of available molecular ecology

and evolutionary genomic studies of species radiations in animals and plants. Many other interesting studies on other groups may be identified by broadening the search to include also taxonomically focused journals, which could be a worthwhile enterprise for future work.

Our graphical summary of reviewed studies (**Fig. 1**) reveals an upward trend in yearly numbers of molecular ecology studies of species radiations over the last 20 years. In particular, a rapid increase in studies situated within world biodiversity hotspots becomes apparent from the early 2000's onwards (**Fig. 1**), coinciding roughly with the publication of major synthetic work on the role of biodiversity hotspots in setting world-wide conservation priorities (Myers *et al.* 2000). Our review database also allowed cross-tabulation of the reviewed articles with taxonomic groups, biogeographic features, and molecular methods covered by each study (**Table S1**). Perhaps most conspicuously, the distribution of studies was highly unequal across taxonomic groups (**Fig. 2**; Chi square test; $p<0.001$). Studies focusing on fishes were greatly overrepresented, whereas several other groups of organisms re grossly under-represented, including plants, arthropods, and fungi (**Fig. 2**). This attests to the important role of fish model systems (e.g. cichlids and sticklebacks) in inspiring and driving progress in molecular ecology / population genetic research on species radiations, and it points to the need for further work including also other species-rich and ecologically important organismal groups. The reviewed studies were also unequally distributed among biogeographic regions ($p<0.001$), with a strong representation of research in the tropics (49 dies), followed by temperate regions (20 studies) and regions with Mediterranean mates (9 studies) (**Table 1**; **Table S1**). This all makes sense, as it indicates molecular ecologists focused their research on regions with the highest species richness (the tropics), regions with traditionally strong research funding and infrastructure (temperate Europe and North America), and regions that "tick both boxes" (biodiversity hotspots with Mediterranean climates).

Examination of the molecular marker methods used in each study (**Box 1**) indicated that molecular ecology studies of radiations carried out thus far relied on organellar (plastid and mitochondrial) DNA sequences, microsatellite markers, and Sanger-sequenced nuclear

genes or genome regions (60, 28, and 26 studies, respectively; **Table 1**; **Table S1**). The preponderance of organellar studies was expected given that sequencing of organellar DNA regions is widely known as the simplest approach for gathering genetic data on many species, an advantage that is also appreciated by the DNA barcoding community (Hebert *et al.* 2003; Collins & Cruickshank 2013). Interestingly, a sizable proportion of articles (13 dies representing 14% of all reviewed papers) already used NGS genotyping methods such as Restriction site Associated DNA sequencing (RAD-seq) or Genotyping by Sequencing (GBS) for studying radiations. This indicates great potential for molecular ecologists to adopt short-read genotyping-by-sequencing methods for their research on iations, especially for radiating complexes of closely related species and sub-specific taxa, where *de novo* clustering or reference-mapping of sequence reads is expected to be easiest (**Box 1**). The successful integration of GBS and RAD-seq into studies of radiations was clearly facilitated by the development of user-friendly methods for *de novo* clustering of sequence reads (e.g. Catchen *et al.* 2013), and by the availability of genome assemblies for reference-mapping and detecting DNA polymorphisms in model radiations often used by evolutionary biologists (The Heliconius Genome Consortium 2012; Brawand *et al.* 2014; Christe *et al.* 2016; Varikova *et al.* 2016). There is considerable potential to extend this type of research to polyploids and taxa with variation in ploidy level, provided that appropriate statistical tools are used to detect and analyze genetic loci and allelic variation (e.g. Arnold *et al.* 2016; Zohren *et al.* 2016). Although perhaps of less concern in animals and fungi, variation in ploidy is significant in many plant lineages (Soltis & Soltis 2016). Thus, NGS approaches to studying polyploid groups may expand or even change our concepts of species diversity, radiations, and global patterns of diversity. This research will no doubt benefit greatly from the continued improvement of read lengths in high-throughput sequencing technologies (e.g. PacBio or Oxford Nanopore), which will aid the identification of homologues and paralogues arising from genome doubling.

As outlined earlier, the trade-off between sampling many individuals, populations, and species from radiating clades and sampling many genetic markers from their genomes represents a major limitation in studies of radiations. Sufficient sampling is necessary to confidently capture the genomic variation present within and among radiating taxa. Simply put, this is important for assessing the influence of population-level processes on macro-olutionary patterns. To provide an illustrative example familiar to most researchers dealing with diversification and radiation, molecular genetic data can potentially challenge the *a priori* taxonomic delimitation of species, the fundamental biological units of investigation in most dies of radiations, and the ability to do so will depend on sample size. We were thus interested in comparing *prior* numbers of species (=*a priori* species designations based on taxonomic knowledge) to *posterior* numbers of species (=after applying molecular markers) across the reviewed studies (**Fig. 3**). Our assignment of 'species' in both cases followed the verdicts by the authors of the reviewed studies, backed up by quantitative data presented in reviewed papers; we acknowledge that this procedure may be affected by philosophical predispositions of each study´s authors concerning species delimitation.

We found numerous departures from a perfect match between prior and posterior ecies numbers (=departures from the diagonal), especially for studies that focused all their efforts on a small prior number of species (≤10) (**Fig. 3**). These departures suggest that molecular ecologists either "lumped" species that had been hypothesized to be distinct at the outset of their studies, or that they alternatively detected new cryptic taxa based on the molecular genetic data. We modelled the difference between prior and posterior species numbers with a range of different predictor variables using generalized linear mixed effect models (GLM's), making use of the glm, lme, and MuMin packages in R (**Table S2**). Following initial exploratory trials including all possible predictor variables in model comparisons, we focused our analysis on the effects of the type of genome sampled (nuclear vs. organellar, extracted from **Table S1)**. This was justified because this variable showed the highest importance among all predictor variables (**Table S2**). We also examined the effect of sample size expressed as the number of populations per species (no. pop) and

number of individuals per population (ind_per_pop) along with the type of genome sampled, as sample size can significantly influence the ability to detect statistical relationships. The results revealed a consistent and significant effect of the type of genome sampled, with nuclear genomic data leading to the detection of more species compared to organellar data alone (**Table S2**; model 5; slope=1, $p<0.01$, model AIC=342.85). With regard to sample size, number of populations led to a decrease in the residual sum of squares and stronger model support (**Table S2**; chi-square model 1 ~ model 5: residual deviance=7.23, p=0.07, ΔAIC=1.31). We note that other, untested factors may in principle also have influenced these patterns, such as the size of "manageable" species complexes. For example, larger groups may already be maximally split by taxonomists, whereas smaller groups could present more uncertainty in species numbers with more room for "lumping" or "splitting". Nevertheless, an important role of sample size is difficult to dispute (**Table S2**). Taken together, our results suggest that increasing the numbers of populations sampled for each species facilitates the detection of cryptic taxa in molecular ecology studies of radiations, but the choice between genomic vs. organellar DNA is far more important. This clearly calls for an increased sampling from nuclear genomes to fully document and understand species radiations.

Recent achievements and current research challenges

spite recent ground-breaking research on a small number of 'model' radiations in animals and plants (e.g. The Heliconius Genome Consortium 2012; Brawand *et al.* 2014; Lamichhaney *et al.* 2015; Novikova *et al.* 2016) and the many interesting studies analyzed in the present contribution, it is clear that most students and researchers of radiations in non-model groups still face considerable challenges putting together micro- and macroevolutionary approaches and data to obtain a more complete picture of the drivers and limits of radiations at all relevant spatial and temporal scales. This already becomes apparent from the observation that most studies reviewed here used either a population genetic sampling strategy (very few species, many individuals per species) or a phylogenetic sampling strategy (many species, very few individuals per species). Thus, the numbers of

species sampled in each study and numbers of individuals sampled for each population were negatively correlated (Spearmans rho = -0.439, p<0.0001). Hence, in our opinion only few of the reviewed studies managed to convincingly cross the micro / macro divide. This includes the studies by Cavender-Bares *et al.* (2015) on American live oaks (*Quercus* spp.), Nadeau *et al.* (2013) on *Heliconius* butterflies, the comparative landscape genetics of Darwin´s finches by Petren *et al.* (2005), several of the cichlid papers, and Prunier *et al.* (2010) on the southern African plant radiation of Proteas. The latter study presents a rare test for ´explosive bursts´ of speciation as expected for early stages of adaptive radiation (Gavrilets & Vose 2005), making use of hierarchical variance partitioning of molecular

netic marker data.

Overall, our targeted review of molecular ecology studies of radiations in one of the leading subject journals suggests that much progress has been made in recent years.

veral studies achieved extensive sampling of nuclear genomes and transcriptomes using NGS approaches and compared the results to phenotypic diversity in radiating taxa, thus

ealing mosaics of adaptive / parallel and neutral / nonparallel patterns of evolution (e.g. Keller *et al.* 2013; Manousaki *et al.* 2013; Nadeau *et al.* 2013). Such mosaic patterns have long been predicted for ecological speciation and adaptive radiation in the face of gene flow (Gavrilets & Vose 2005; Nosil 2012). Studies such as this now set the stage for future work to elucidate the mechanisms and drivers of radiation in a much greater number of

ologically important radiations of animals, plants, fungi, and other organisms (**Fig. 2**). Clearly, far more nuclear genomic data (in addition to information on organellar genomes) will be necessary to resolve both patterns and processes responsible for radiations in a

aningful way. In fact, using the scatter plot in **Fig. 3** as an illustrative example, we encourage aspiring students and researchers studying radiations to face the challenge of increasingly pushing their research efforts from the lower left towards the upper right corner of the graph. This implies sampling more species with stronger within-species sampling (especially more populations of each species) and more elaborate sampling of nuclear

genomes. How can this goal possibly be reached at reasonable effort and cost? Below we suggest possible ways forward for the coming years, using a graphical approach to sketch current challenges and opportunities (**Fig. 4**), and drawing from the 'toolbox' now available to lecular ecologists (**Box 1**).

Opportunities and priorities for future research

In our view, possibly the greatest opportunity and priority for future research on species radiations in the coming years will reside in scaling up the sampling and analysis of populations, species, and genomes of radiating taxa across all relevant evolutionary time scales, in order to bridge the traditional micro / macro divide in studies of radiations alluded to in the introduction of this paper. We sketch this task graphically in **Fig. 4**. It is now widely accepted that understanding the causes of radiations requires the in-depth analysis of ecological and biogeographical correlates of diversification at the macro-evolutionary scale (Barraclough & Vogler 2000; Linder 2008; Cavender-Bares *et al.* 2009; Kozak & Wiens 2010). This is readily illustrated by research on niche conservatism and niche evolution (Wiens & Donoghue 2004). **Fig. 4A** shows phylogenetic trees and networks of species and sub-specific taxa from a hypothetical radiation of animals or plants. Since the authors of this per study neotropical plant radiations, we picked an example of taxa radiating on the South American continent. The orange and green circles at the tips of the tree superimposed on the geographic map (**Fig. 4A)** represent distinct ecological traits, e.g. green to orange y indicate a shift to the epiphytic growth habit or to hummingbird pollination in neotropical plants, that is, a key innovation facilitating the invasion of a new adaptive zone (Givnish 2015). We note that the graph represents a simplification, as the invasion of new niches or aptive zones will often be facilitated by changes in multiple traits, rather than a single trait alone. Nevertheless, the graph may serve to illustrate our point: the orange trait is sociated with more rapid diversification, thus its appearance – and niche evolution associated with it – may be hypothesized to be causally linked to rapid diversification in this

group (Wiens & Donoghue 2004; Givnish 2015). Still, testing causality and the actual evolutionary and molecular mechanisms responsible requires additional research based on population genetics, genomics, and molecular biology.

The inset under the magnifying glass in **Fig. 4A** illustrates the population geneticist's approach of "looking inside the tips of the tree" of a radiation (e.g. *sensu* Chaves *et al.* 2016) and uncovering key genetic patterns and processes for radiating taxa at the sub-specific

el, e.g. using tree- or network-based approaches. Molecular ecologists generally seek to support such results by quantitative data on the hierarchical partitioning of genomic variation around the species level. Here, yellow, orange, and red circles indicate locally adapted populations or ecotypes with differences in adaptive traits with known or unknown genetic basis. Population divergence may be due to drift or locally varying selection on adaptive traits, and the latter scenario is depicted here. A well developed framework exists for detecting the footprints of selection in molecular population genetic or quantitative trait data (Conner & Hartl 2004; Nielsen 2005), and readers of this journal will be well familiar with these concepts. What matters most to the present contribution – and to the future of research on radiations in our view – is to break down the traditional barriers between the macro- and micro-scales depicted in **Fig. 4A**. Below we argue that this is now becoming

sible in many non-model radiations of animals and plants. We place particular emphasis on practical aspects of gathering the necessary genomic data, rather than theoretical or analytical issues, which have been dealt with elsewhere (above).

To appreciate the current state-of-the-art of molecular ecology research on evolutionary radiations, it helps to remember that the most widely used molecular tools used

researchers studying radiations thus far (**Box 1**) are informative and useful either at very short evolutionary time scales, i.e. within and between closely related species, or at rather long evolutionary time scales, i.e. across many species or higher-level taxa (**Fig. 4B)**. Microsatellites and RAD-seq / GBS clearly fall within the former group, whereas sequencing

organellar markers generally falls within the latter (**Box 1**; **Fig. 4B)**, perhaps with the exception of synonymous mutations in mitochondrial DNA regions under weak selective

constraints (Hebert *et al.* 2003). All these approaches will likely continue to be useful for studies of specific aspects of radiations, and their general potentials and pitfalls have been cussed elsewhere (e.g. Barbará *et al.* 2007; Davey *et al.* 2011; Lexer *et al.* 2013; Ellegren 14; Schlötterer *et al.* 2014). Nevertheless, the future of studies of radiations clearly resides in other approaches able to span a larger range of evolutionary time scales. Thus, aspiring students and researchers may wish to give priority to these, if the goal is to understand the mechanisms underlying diversification across the micro / macro divide, that is, across the entire sequence of events from population divergence to speciation to radiation.

Whole genome sequencing (WGS) of radiating taxa emerges as potentially the most powerful and desirable approach to connect research on micro- and macroevolutionary aspects of radiations within or between related genera (**Box 1**; **Fig. 4B**). In fact, a growing number of studies demonstrate that applying WGS to studies of speciation and radiation is entirely feasible, as long as an assembled and annotated reference genome and sufficient resources are available (The Heliconius Genome Consortium 2012; Brawand *et al.* 2014; Lamichhaney *et al.* 2015; Ellegren 2014; Christe *et al.* 2016; McGee *et al.* 2016; Novikova *et al.* 2016). Although not covered specifically in our study, WGS approaches are also certainly sible in species groups originating from genome doubling, with available case studies spanning from recent autopolyploids (Arnold *et al.* 2016) to allopolyploids (products of past hybridization events; Zohres *et al.* 2016) to paleopolyploids with ancient traces of whole genome duplications (e.g. Christe *et al.* 2016). This is good news for students of organismal groups in which genome doubling has evidently contributed to diversification, which includes ny families of flowering plants (Soltis & Soltis 2016). In all these contexts, it helps to keep in mind that the human power, creativity, and time required to develop and validate the nstantly improving bioinformatics pipelines needed tends to be a far more limiting resource than the money spent on the actual sequencing. In our experience, this fact is sometimes still under-appreciated by research groups looking to venture into second- or third-generation sequencing approaches for studying radiations. Given sufficient, well-trained human power for bioinformatics and downstream evolutionary genetics we predict that WGS will contribute

greatly to future studies of radiations, as more annotated reference genome assemblies become available for non-model groups, and as sequencing throughput continues to increase dramatically (Davey *et al.* 2011). In effect, we anticipate a smooth transition between the low-coverage ´genome skimming´ approaches already widely applied in systematic biology (Straub *et al.* 2012; Hollingsworth *et al.* 2016; **Box 1**) and full-blown WGS, as sequencing costs decrease further in the coming years. Thus, beginning students and researchers interested in studying radiations (or any other genetic aspect of biodiversity) at the present time are well advised to acquire vital skills in genome bioinformatics early on in their careers, along with a firm understanding of evolutionary biology and the ability to analyze genetic information jointly with extensive datasets on multivariate phenotypes, niche space, and fitness landscapes.

As an alternative to WGS, targeted resequencing of selected genome regions yields more manageable datasets for potentially much larger sample sizes, which is highly desirable for studies aiming to span both micro- and macro-evolutionary scales. Thus, we spect that many researchers studying radiations will wish to turn to targeted resequencing as an attractive and feasible alternative to WGS. Transcriptome sequencing (RNA-seq) has also yielded valuable insights in studies of ecotype divergence, speciation, and radiation, as exemplified by two cichlid papers among the reviewed studies (Colombo *et al.* 2013; Manousaki *et al.* 2013). Nevertheless, due to the difficulty of sampling fresh material for proper RNA conservation and extraction, we suspect that future studies of radiations may h to employ RNA-seq primarily as a marker discovery tool for subsequent exome (i.e. protein coding DNA) capture.

Approaches for targeted resequencing (‘sequence capture’ from here onwards) have been in place for several years (**Box 1**; Mamanova *et al.* 2010; Meyer & Kircher 2010). Sequence capture has been applied successfully to multi-species settings including iations (Lemmon *et al.* 2012; Nadeau *et al.* 2012), and clever indexing / multiplexing strategies enable standard second-generation (e.g. Illumina) sequencing of thousands of samples (Rohland & Reich 2012). Notably, sequence capture is known to be practicable

even for biological samples yielding only minute amounts of DNA, even if partially degraded (Mamanova *et al.* 2010; Meyer & Kircher 2010). This is relevant for the study of 'recalcitrant' non-model species posing difficulties during DNA extraction, and even more so for projects based on biological material from herbaria and museum collections (Buerki & Baker 2016). We anticipate that herbaria (and museum collections more generally) will play crucial roles in facilitating research on broad-scale patterns of genomic diversity and diversification, given their richness of preserved specimens that yield fragmented DNA suitable for NGS techniques. Herbaria and other collections thus hold great promise for enabling or expanding nomic studies of species that may be rare and/or protected in the wild.

ncluding remarks

Among currently available molecular ecology approaches (**Box 1** and **Fig. 4B)**, WGS and sequence capture may be best suited for addressing the current major research gaps and challenges faced by studies of species radiations, which can be summarized concisely as follows: (1) to tackle the many 'non-model' organismal groups currently underrepresented in dies of radiations (**Fig. 2**), including those for which high-quality tissue samples are icult to come by or specimens from herbaria or museum collections (Suchan *et al.* 2016), (2) to scale up the sampling of populations and species in order to move the field forward from 'speciation genomics' towards 'evolutionary radiation genomics', thus filling the sparsely populated upper right portion of sample size space exemplified in **Fig. 3**, (3) to achieve more elaborate and representative sampling of nuclear genomes in studies of radiations, (4) to increasingly capture those genes and regulatory elements that matter most to the evolutionary process, in addition to neutrally evolving sequence regions. The last goal can achieved by picking the 'bait' molecules used for target sequence capture to include sets of candidate genes (best with surrounding regulatory regions) and pathways with clear *a priori* hypotheses about causal relationships with diversification (The Heliconius Genome Consortium 2012; Lamichhaney *et al.* 2015, Eyres *et al.* 2016), e.g. candidate genes for adaptive traits or 'key innovations' in the case of adaptive radiation. With transcriptome and

genome assemblies accumulating rapidly for many species and swift progress in the development of functional annotation tools, it seems that the time is now right for molecular

ologists to accept the challenge of studying radiations (as opposed to studying pairs or small groups of diverging taxa), thus building on theoretical advances in connecting micro- and macro-evolutionary studies made in recent years (Salamin *et al.* 2010; Smadja & Butlin 2011; The Marie Curie SPECIATION network 2011; Seehausen *et al.* 2014; Simões *et al.*

16; Kostikova *et al.* 2016). The role of transposable element (TE) dynamics (Brawand *et al.* 2014), structural genomic (Feulner *et al.* 2013) and epigenomic variation in fuelling radiations also represent highly promising avenues for future research. We look forward to seeing many exciting new studies of radiations that successfully bridge micro- and macro-evolutionary approaches in the coming years.

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ta accessibility

sential features of all reviewed studies are enclosed as Supporting Information (SI) Table S1, and all 93 references are included in the reference section of the paper.

Author Contributions

MdlH, CL, and MP conceived the study, MdlH, DNK, CL, and MP analyzed literature data, and MdlH and CL wrote the paper with input from all coauthors.

Box 1. Genomic approaches suitable for studying evolutionary radiations

Nuclear microsatellites

Nuclear microsatellite markers have been utilized for studies of population genetic diversity and differentiation since the early 1990´s (Jarne & Lagoda 1996). The sequence regions flanking microsatellites are often sufficiently conserved for designing PCR primers that are functional across related species and sometimes even across genera (Schlötterer *et al.* 1991; Steinkellner *et al.* 1997; Barbará *et al.* 2007), thus microsatellite markers have been useful for studies of species complexes and radiations. This is exemplified by 27 of our reviewed studies that used microsatellites as molecular tools for studying radiations (**Table 1; Table S1**). Due to their complex mutation patterns and a low potential for multiplexing (i.e. moderate cost efficiency), many molecular ecologists currently tend to prefer other methods for studying radiations (below). We suspect that microsatellites will continue to be useful for understanding fine-scale patterns of population subdivision and/or variation in reproductive strategies in radiating taxa, and generally in situations where low sequence polymorphism calls for markers with high mutation rates.

AFLP

Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.* 1995) is a DNA fingerprinting method that was in frequent use mainly in the 1990's and early 2000's. The presence / absence patterns in AFLP are typically interpreted as dominant marker information. The method has been useful for studying radiations (e.g. six of our reviewed studies used it) and has largely been replaced by NGS-based genotyping by sequencing approaches in recent years (below).

Amplicon sequencing of organellar DNA

Amplicon sequencing of organellar (i.e. mitochondrial or plastid) DNA has long been popular in phlyogeographic and phylogenetic studies (Zhang & Hewitt 2003), mainly because of the ease of discovering and detecting polymorphism in organellar genomes. Because organellar

genomes offer both conserved regions for PCR primer design and variable regions to be used as markers, they continue to be used for biogeographic and DNA barcoding studies (Hebert *et al.* 2003; Collins & Cruickshank 2013). The advantages of organellar markers lie in their facile detection across large evolutionary distances, the haploid (=simple) nature of their sequence data, and their generally maternal mode of inheritance, which provides information on the dispersal biology of maternal lineages (e.g. seed dispersal in the case of flowering plants). Their main disadvantage lies in the fact that in general, only a single non-recombining locus is analyzed. Sixty of our reviewed studies used organellar markers, which attests to their popularity in molecular ecology studies of radiations. Twenty of these relied on organellar genomes in the complete absence of nuclear genomic data. We predict that organellar markers will continue to be useful in studies of radiations in conjunction with nuclear markers, especially for understanding the dispersal biology of radiating species.

Amplicon sequencing of selected nuclear genes

Amplicon sequencing of nuclear genes has gained rapidly in popularity in recent years because of its obvious advantages, e.g. gaining access to bi-parentally inherited nuclear genomic information across multiple species at reasonable cost. Amplicon sequencing of nuclear DNA was greatly facilitated by the ability to detect orthologous single or low copy nuclear genes in comparative genomic databases (e.g. Duarte *et al.* 2010) and the rapid development of the analytical toolbox available for analyzing sequence data (Nielsen 2005) including rapid haplotype phasing. In fact, 26 of our reviewed studies made use of amplicon sequencing of nuclear loci, typically using the classical Sanger method. We suspect that amplicon sequencing of nuclear genes will continue to be useful for medium-throughput studies of radiations, especially if carried out using cost-effective sequencing technologies. In this context, an advantage of amplicon sequencing compared to targeted resequencing (below) is its greater ability to recover homologous sequence information in the face of tial or whole-genome duplications.

NGS genotyping using GBS or RAD-seq approaches

Genotyping by sequencing approaches based on second or later generation sequencing technologies are rapidly increasing in popularity in studies of radiations, as exemplified by 13 recent studies in our review database (14% of studies) that used them (**Table 1; Table S1**). Typical protocols include Restriction site Associated DNA sequencing (RAD-seq; Baird *et al.* 2008), Genotyping by Sequencing (GBS; Elshire *et al.* 2011; Parchman *et al.* 2012), or double digest RAD sequencing (ddRADseq; Peterson *et al.* 2012). All of these involve the idea of reducing the complexity of sequencing templates by restriction digestion (van Orsouw *et al.* 2007), followed by different selection steps and the addition of barcoding molecules to allow multiplexing on high-throughput (e.g. Illumina) sequencing platforms. RAD-seq approaches contain shearing and size selection steps not present in GBS (Davey *et al.* 2011), and ddRAD-seq was especially designed to achieve more even sequencing coverage across individuals and genomes (Peterson *et al.* 2012). The general advantages and disadvantages of these approaches have been discussed elsewhere (e.g. Davey *et al.* 2011; Peterson *et al.* 2012; Puritz *et al.* 2014). In the context of studies of radiations, we expect that RAD and GBS methods will continue to be useful in the coming years. Their potential is greatest for complexes of closely related taxa, where sequence divergence is still low enough to allow reliable alignment during *de novo* clustering ("stack building") or during mapping of sequence reads against reference genomes. This is important because both *de novo* clustering and reference-mapping require the definition of sequence mismatch thresholds (e.g. 95% criterion or maximum of 3 mismatches) to maximize the chance that similar sequence reads obtained from different populations and species are indeed allelic, i.e. that they are orthologous rather than paralogous (see Davey *et al.* 2011; Lexer *et al.* 2013). With these caveats in mind, RAD and GBS approaches represent highly valuable sources of many thousands of codominant, sequence-based genetic markers for studying radiations, with good potential to target gene-rich genome regions by choosing methylation-sensitive restriction endonucleases.

Whole genome sequencing (WGS)

Currently available sequencing technologies make it feasible to study the genomes of radiating taxa by resequencing of entire genomes at low coverage (typically 10-20x). At the present time, this is most commonly done within larger research projects or consortia (The Heliconius Genome Consortium 2012; Brawand *et al.* 2014; Lamichhaney *et al.* 2015; Ellegren 2014; Novikova *et al.* 2016). Nevertheless, WGS studies of radiating taxa are also entirely feasible for smaller research groups and are now within reach for molecular ecology studies of radiations in many groups of animals and plants, as long as an assembled reference genome is available in the same family or preferably the same genus. For example, a back-of-the-envelope calculation shows that for an organism with a genome of 500 mega bases (MB), 100 individuals can be individually sequenced at >10x coverage by current second-generation sequencing technologies (e.g. Illumina HiSeq) for the equivalent of 25 000-35 000 US dollars or less. Thus, WGS studies of radiations are primarily limited by the availability of suitable biological samples to represent the 'genomic substrate' present in the target radiation and the necessary computational skills for analyzing the data, rather than the actual sequencing step. There is great potential to further increase sample sizes in WGS studies of radiations by sequencing and analyzing pools of DNA, with each pool consisting of many individuals from particular populations, locality, or taxa (Schlötterer *et al.* 2014). In effect, we suspect that WGS will increasingly replace so-called ´genome skimming´ approaches often used in systematic biology (very low coverage sequencing yielding mainly plastid, mitochondrial, and ribosomal DNA data; Straub *et al.* 2012) as sequencing costs decrease further and more nuclear reference genome assemblies become available. Thus, we expect that low-coverage WGS of populations and species will rapidly become the nchmark of population genetic and molecular ecology studies of radiations.

Targeted resequencing

Targeted resequencing or 'sequence capture' is an increasingly popular approach of partial genomic resequencing. Its main rationale is to focus sequencing efforts on a manageable number of genome regions of interest (e.g. 'exome capture' or resequencing of specific loci),

rather than the entire genome. Approaches for sequence capture have been in place for several years now, and currently used methods (either in solution or on arrays) greatly outperform conventional PCR amplicon sequencing in terms of throughput, efficiency, and practicability (Mamanova *et al.* 2010; Meyer & Kircher 2010). Thus, sequence capture is idly becoming the method of choice for evolutionary genetic studies from the micro- to the macro-scale (Rohland & Reich 2012; Lemmon *et al.* 2012; Nadeau *et al.* 2012). We expect that targeted resequencing will greatly help students and researchers of species radiations to overcome the challenge of sequencing many individuals, populations, and species for fairly large numbers of functionally interesting genome regions. Since they effectively enrich genomic DNA templates for the chosen targets, capture approaches are expected to be useful for recovering genomic information from tissues that contain only minute amounts of DNA, as is typically the case for museum or herbarium specimens (Buerki & Baker 2016). Thus, sequence capture holds great promise for the rapid assessment of genomic diversity across a wide range of spatial, temporal, and taxonomic scales.

References

Abbott R, Albach D, Ansell S *et al.* (2013) Hybridization and speciation. *Journal of Evolutionary Biology*, **26**, 229–246.

ertson RC, Powder KE, Hu Y *et al.* (2014) Genetic basis of continuous variation in the levels and modular inheritance of pigmentation in cichlid fishes. *Molecular Ecology*, **23**, 5135–5150.

Andrews KR, Perrin WF, Oremus M *et al.* (2013) The evolving male: Spinner dolphin (*Stenella longirostris*) ecotypes are divergent at y chromosome but not mtDNA or autosomal markers. *Molecular Ecology*, **22**, 2408–2423.

Arnold ML (2016) Divergence with genetic exchange, *Oxford University Press*, Oxford.

Arnold BJ, Lahner B, DaCosta JM, Weismann CM, Hollister JD, Salt DE, Bomblies K, Yant L (2016) Borrowed alleles and convergence in serpentine adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 8320–8325

Austin JJ, Arnold EN, Bour R (2003) Was there a second adaptive radiation of giant tortoises in the Indian Ocean? Using mitochondrial DNA to investigate speciation and biogeography of Aldabrachelys (Reptilia, Testudinidae). *Molecular Ecology*, **12**, 1415–1424.

Baird NA, Etter PD, Atwood TS *et al.* (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE*, **3**.

Barbará T, Palma-Silva C, Paggi GM *et al.* (2007) Cross-species transfer of nuclear microsatellite markers: Potential and limitations. *Molecular Ecology*, **16**, 3759–3767.

rluenga M, Meyer A (2004) The Midas cichlid species complex: Incipient sympatric speciation in Nicaraguan cichlid fishes? *Molecular Ecology*, **13**, 2061–2076.

Barraclough T, Vogler A (2000) Detecting the Geographical Pattern of Speciation from Species-Level Phylogenies. *The American Naturalist*, **155**, 419–434.
Bastos-Silveira C, Santos SM, Monarca R, Mathias M da L, Heckel G (2012) Deep mitochondrial introgression and hybridization among ecologically divergent vole species. *Molecular Ecology*, **21**, 5309–5323.
Beheregaray LB, Sunnucks P (2001) Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. *Molecular Ecology*, **10**, 2849–2866.
Berner D, Roesti M, Hendry AP, Salzburger W (2010) Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Molecular Ecology*, **19**, 4963–4978.
Bonett RM, Chippindale PT (2004) Speciation, phylogeography and evolution of life history and morphology in plethodontid salamanders of the *Eurycea multiplicata* complex. *Molecular Ecology*, **13**, 1189–1203.
Bracken FSA, Hoelzel AR, Hume JB, Lucas MC (2015) Contrasting population genetic structure among freshwater-resident and anadromous lampreys: the role of demographic history, differential dispersal and anthropogenic barriers to movement. *Molecular Ecology*, **24**, 1188–1204.
Brawand D, Wagner CE, Li YI *et al.* (2014) The genomic substrate for adaptive radiation in African cichlid fish. *Nature*, **513**, 375–381.
Buerki S, Baker WJ (2016) Collections-based research in the genomic era. *Biological Journal of the Linnean Society*, **117**, 5–10.
Butlin R, Debelle A, Kerth C *et al.* (2012) What do we need to know about speciation? *Trends in Ecology and Evolution*, **27**, 27–39.
Campagna L, Gronau I, Silveira LF, Siepel A, Lovette IJ (2015) Distinguishing noise from signal in patterns of genomic divergence in a highly polymorphic avian radiation. *Molecular Ecology*, **24**, 4238–4251.
Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: An analysis tool set for population genomics. *Molecular Ecology*, **22**, 3124–3140.
Cavender-Bares J, González-Rodríguez A, Eaton DAR *et al.* (2015) Phylogeny and biogeography of the American live oaks (Quercus subsection Virentes): a genomic and population genetics approach. *Molecular Ecology*, **24**, 3668–3687.
Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW (2009) The merging of community ecology and phylogenetic biology. *Ecology Letters*, **12**, 693–715.
Chakrabarty P, Davis MP, Smith WL, Baldwin ZH, Sparks JS (2011) Is sexual selection driving diversification of the bioluminescent ponyfishes (Teleostei: Leiognathidae)? *Molecular Ecology*, **20**, 2818–2834.
Charlesworth B, Charlesworth D (2016) Population genetics from 1966 to 2016. *Heredity*, 1–8.
Chaves J, Cooper E, Hendry A, et. al. (2016) Genomic variation at the tips of the adaptive radiation of Darwin's finches. *Molecular Ecology,* **25**, 5282-5295.
Christe C, Stölting KN, Paris M. *et al.* (2016). Adaptive evolution and segregating load contribute to the genomic landscape of divergence in two tree species connected by episodic gene flow. *Molecular Ecology*, published online early, doi: 10.1111/mec.13765.
Collins RA, Cruickshank RH (2013) The seven deadly sins of DNA barcoding. *Molecular Ecology Resources*, **13**, 969–975.
Colombo M, Diepeveen ET, Muschick M *et al.* (2013) The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes. *Molecular Ecology*, **22**, 670–684.
Conner JK, Hartl DL (2004) A Primer of Ecological Genetics, *Sinauer Associates,* Sunderland, MA.
Cox SC, Prys-Jones RP, Habel JC, Amakobe BA, Day JJ (2014) Niche divergence promotes rapid diversification of East African sky island white-eyes (Aves: Zosteropidae). *Molecular Ecology*, **23**, 4103–4118.
Dalman K, Oslon Å, Stenlid J (2010) Evolutionary history of the conifer root rot fungus Heterobasidion annosum sensu lato. *Molecular Ecology*, **19**, 4979–4993.

Davey JWJ, Hohenlohe PA, Etter PPD *et al.* (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, **12**, 499–510.
De Busschere C, Handrickx F, Van Belleghem SM *et al.* (2010) Parallel habitat specialization within the wolf spider genus Hogna from the Galápagos. *Molecular Ecology*, **19**, 4029–4045.
Deagle BE, Jones FC, Absher DM, Kingsley DM, Reimchen TE (2013) Phylogeography and adaptation genetics of stickleback from the Haida Gwaii archipelago revealed using genome-wide single nucleotide polymorphism genotyping. *Molecular Ecology*, **22**, 1917–1932.
Derome N, Duchesne P, Bernatchez L (2006) Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. *Molecular Ecology*, **15**, 1239–1249.
rycke S, Remerie T, Backeljau T *et al.* (2008) Phylogeography of the *Rhabditis (Pellioditis) marina* species complex: evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. *Molecular Ecology*, **17**, 3306–3322.
wle EJ, Morgan-Richards M, Brescia F, Trewick SA (2015) Correlation between shell phenotype and local environment suggests a role for natural selection in the evolution of Placostylus snails. *Molecular Ecology*, **24**, 4205–4221.
Duarte JM, Wall PK, Edger PP *et al.* (2010) Identification of shared single copy nuclear genes in *Arabidopsis*, *Populus*, *Vitis* and *Oryza* and their phylogenetic utility across various taxonomic levels. *BMC Evolutionary Biology*, **10**, 61.
Ellegren H (2014) Genome sequencing and population genomics in non-model organisms. *Trends in Ecology and Evolution*, **29**, 51–63.
Elshire RJ, Glaubitz JC, Sun Q *et al.* (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, **6**.
Emerson BC, Gillespie RG (2008) Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology and Evolution*, **23**, 619–630.
Eyres I, Duvaux L, Gharbi K *et al.* (2016) Targeted re-sequencing confirms the importance of chemosensory genes in aphid host race differentiation. *Molecular Ecology*, published online early: doi: 10.1111/mec.13818.
der JL, Egan SP, Nosil P (2012) The genomics of speciation-with-gene-flow. *Trends in Genetics*, **28**, 342–350.
Feulner PGD, Chain FJJ, Panchal M *et al.* (2013) Genome-wide patterns of standing genetic variation in a marine population of three-spined sticklebacks. *Molecular Ecology*, **22**, 635–649.
Flaxman SM, Wacholder AC, Feder JL, Nosil P (2014) Theoretical models of the influence of genomic architecture on the dynamics of speciation. *Molecular Ecology*, **23**, 4074–4088.
Foote AD, Newton J, Piertney SB, Willerslev E, Gilbert MTP (2009) Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations. *Molecular Ecology*, **18**, 5207–5217.
rd AGP, Dasmahapatra KK, Rüber L *et al.* (2015) High levels of interspecific gene flow in an endemic cichlid fish adaptive radiation from an extreme lake environment. *Molecular Ecology*, **24**, 3421–3440.
Garb JE, Gillespie RG (2009) Diversity despite dispersal: colonization history and phylogeography of Hawaiian crab spiders inferred from multilocus genetic data. *Molecular Ecology*, **18**, 1746–1764.
Garrick RC, Benavides E, Russello MA *et al.* (2014) Lineage fusion in Galápagos giant tortoises. *Molecular Ecology*, **23**, 5276–5290.
vrilets S, Vose A (2005) Dynamic patterns of adaptive radiation. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18040–18045.
Genner MJ, Knight ME, Haesler MP, Turner GF (2010) Establishment and expansion of Lake Malawi rock fish populations after a dramatic Late Pleistocene lake level rise. *Molecular Ecology*, **19**, 170–182.

Givnish T J (2015). Adaptive radiation versus 'radiation' and 'explosive diversification': why conceptual distinctions are fundamental to understanding evolution. *New Phytologist*, **207**, 297-303.
Givnish TJ (2010) Ecology of plant speciation. *Taxon*, **59**, 1329–1366.
Glor RE, Losos JB, Larson A (2005) Out of Cuba: overwater dispersal and speciation among lizards in the Anolis carolinensis subgroup. *Molecular Ecology*, **14**, 2419–2432.
Glor RE (2010) Phylogenetic insights on adaptive radiation. *Annual Review on Ecology, Evolution and Systematics*, **41**, 251-270.
Gow JL, Peichel CL, Taylor EB (2006) Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Molecular Ecology*, **15**, 739–752.
Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences*, **270**, 313–21.
Hendrickx F, Backeljau T, Dekoninck W *et al.* (2015) Persistent inter- and intraspecific gene exchange within a parallel radiation of caterpillar hunter beetles (Calosoma sp.) from the Galápagos. *Molecular Ecology*, **24**, 3107–3121.
key AJR, Lavery SD, Hannan DA, Baker CS, Clements KD (2009) New Zealand triplefin fishes (family Tripterygiidae): contrasting population structure and mtDNA diversity within a marine species flock. *Molecular Ecology*, **18**, 680–696.
ding B, Michel E, Natyaganova A V., Sherbakov DY (2003) Molecular evidence reveals a polyphyletic origin and chromosomal speciation of Lake Baikal's endemic asellid isopods. *Molecular Ecology*, **12**, 1509–1514.
llingsworth PM, Li D-Z, van der Bank M, Twyford AD (2016) Telling plant species apart with DNA: from barcodes to genomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **371**, 20150338.
Hulva P, Fornusková A, Chudarkova A *et al.* (2010) Mechanisms of radiation in a bat group from the genus Pipistrellus inferred by phylogeography, demography and population genetics. *Molecular Ecology*, **19**, 5417–5431.
Hurt CR (2004) Genetic divergence, population structure and historical demography of rare springsnails (Pyrgulopsis) in the lower Colorado River basin. *Molecular Ecology*, **13**, 1173–1187.
ra JC, Emerson BC, Richardson DS (2007) Population history of Berthelot's pipit: colonization, gene flow and morphological divergence in Macaronesia. *Molecular Ecology*, **16**, 4599–4612.
Jablonski D (2008) Biotic interactions and macroevolution: Extensions and mismatches across scales and levels. *Evolution*, **62**, 715–739.
Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424–429.
Jordan S, Simon C, Foote D, Englund RA (2005) Phylogeographic patterns of Hawaiian Megalagrion damselflies (Odonata: Coenagrionidae) correlate with Pleistocene island boundaries. *Molecular Ecology*, **14**, 3457–3470.
suga T, White TJ, Koenig G *et al.* (2003) Phylogeography of the fungal pathogen *Histoplasma capsulatum*. *Molecular Ecology*, **12**, 3383–3401.
utt AF, Elmer KR, Meyer A (2012) Genomic signatures of divergent selection and speciation patterns in a "natural experiment", the young parallel radiations of Nicaraguan crater lake cichlid fishes. *Molecular Ecology*, **21**, 4770–4786.
ller I, Wagner CE, Greuter L *et al.* (2013) Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. *Molecular Ecology*, **22**, 2848–2863.
Kidd MR, Kidd CE, Kocher TD (2006) Axes of differentiation in the bower-building cichlids of Lake Malawi. *Molecular Ecology*, **15**, 459–478.
Knowles LL (2008) Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshopprers. *Molecular Ecology*, **10**, 691–701.

Kostikova A, Silvestro D, Pearman PB, Salamin N (2016) Bridging inter- and intraspecific trait evolution with a hierarchical Bayesian approach. *Systematic Biology*, **65**, 417-431.
Kozak KH, Wiens JJ (2010) Accelerated rates of climatic-niche evolution underlie rapid species diversification. *Ecology Letters*, **13**, 1378–1389.
Lamichhaney S, Berglund J, Almén MS *et al.* (2015) Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature*, **518**, 371–375.
wton_Rauh A, Robichaux RH, Purugganan MD (2007) Diversity and divergence patterns in regulatory genes suggest differential gene flow in recently derived species of the Hawaiian silversword alliance adaptive radiation (Asteraceae). *Molecular Ecology*, **16**, 3995–4013.
Lemmon AR, Emme SA, Lemmon EM (2012) Anchored hybrid enrichment for massively high-throughput phylogenomics. *Systematic Biology*, **61**, 727–744.
Linder HP (2008) Plant species radiations: where, when, why? *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **363**, 3097–3105.
Lorenz-Lemke AP, Togni PD, Mader G *et al.* (2010) Diversification of plant species in a subtropical region of eastern South American highlands: a phylogeographic perspective on native Petunia (Solanaceae). *Molecular Ecology*, **19**, 5240–5251.
galhaes IS, Ornelas- Gracia CP, Leal- Cardin M, *et al.* (2015) Untangling the evolutionary history of a highly polymorphic species: introgressive hybridization and high genetic structure in the desert cichlid fish *Herichtys minckleyi*. *Molecular Ecology*, **24**, 4505-4520.
Mamanova L, Coffey AJ, Scott CE *et al.* (2009) Target-enrichment strategies for next-generation sequencing. *Nature Methods*, **7**, 111–118.
Manousaki T, Hull PM, Kusche H *et al.* (2013) Parsing parallel evolution: ecological divergence and differential gene expression in the adaptive radiations of thick-lipped Midas cichlid fishes from Nicaragua. *Molecular Ecology*, **22**, 650–669.
Manthey JD, Klicka J, Spellman GM (2011) Isolation-driven divergence: speciation in a widespread North American songbird (Aves: Certhiidae). *Molecular Ecology*, **20**, 4371–4384.
Marino IAM, Benazzo A, Agostini C *et al.* (2013) Evidence for past and present hybridization in three Antarctic icefish species provides new perspectives on an evolutionary radiation. *Molecular Ecology*, **22**, 5148–5161.
Martin CH, Feinstein LC (2014) Novel trophic niches drive variable progress towards ecological speciation within an adaptive radiation of pupfishes. *Molecular Ecology*, **23**, 1846–1862.
cartney MA, Acevedo J, Heredia C *et al.* (2003) Genetic mosaic in a marine species flock. *Molecular Ecology*, **12**, 2963–2973.
McGee MD, Neches RY, Seehausen O (2016) Evaluating genomic divergence and parallelism in replicate ecomorphs from young and old cichlid adaptive radiations. *Molecular Ecology*, **25**, 260–268.
Mendelson TC, Siegel AM, Shaw KL (2004) Testing geographical pathways of speciation in a recent island radiation. *Molecular Ecology*, **13**, 3787–3796.
Meyer A, Knowles LL, Verheyen E (1996) Widespread geographical distribution of mitochondrial haplotypes in rock-dwelling cichlid fishes from Lake Tanganyika. *Molecular Ecology*, **5**, 341–350.
Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols*, **6**, t5448.
Mims MC, Darrin Hulsey C, Fitzpatrick BM, Todd Streelman J (2010) Geography disentangles introgression from ancestral polymorphism in Lake Malawi cichlids. *Molecular Ecology*, **19**, 940–951.
Morin PA, Parsons KM, Archer FI *et al.* (2015) Geographic and temporal dynamics of a global radiation and diversification in the killer whale. *Molecular Ecology*, **24**, 3964–3979.

Muñoz MM, Crawford NG, McGreevy TJ *et al.* (2013) Divergence in coloration and ecological speciation in the Anolis marmoratus species complex. *Molecular Ecology*, **22**, 2668–2682.
Myers N, Mittermeier R a, Mittermeier CG, da Fonseca G a, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.
deau NJ, Martin SH, Kozak KM *et al.* (2013) Genome-wide patterns of divergence and gene flow across a butterfly radiation. *Molecular Ecology*, **22**, 814–826.
Nadeau NJ, Whibley A, Jones RT *et al.* (2012) Genomic islands of divergence in hybridizing Heliconius butterflies identified by large-scale targeted sequencing. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **367**, 343–53.
NG J, Glor RE (2011) Genetic differentiation among populations of a Hispaniolan trunk anole that exhibit geographical variation in dewlap colour. *Molecular Ecology*, **20**, 4302–4317.
Nice CC, Anthony N, Gelembiuk G, *et al.* (2005) The history and geography of diversification within the butterfly genus Lycaeides in North America. *Molecular Ecology*, **14**, 1741–1754.
Nielsen R (2005) Molecular Signatures of Natural Selection. *Annual Review of Genetics*, **39**, 197–218.
Nosil P. (2012) Ecological speciation. *Oxford University Press*, Oxford.
Novikova, P Y, Hohmann N, Nizhynska V, *et al.* (2016). Sequencing of the genus *Arabidopsis* identifies a complex history of nonbifurcating speciation and abundant trans-specific polymorphism. *Nature Genetics,* **48**, 1077-1082.
Odeen A, Bjorklund M (2003) Dynamics in the evolution of sexual traits: losses and gains, radiation and convergence in yellow wagtails (Motacilla flava). *Molecular Ecology*, **12**, 2113–2130.
Palma-Silva C, Wendt T, Pinheiro F *et al.* (2011) Sympatric bromeliad species (Pitcairnia spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology*, **20**, 3185–3201.
Parchman TL, Benkman CW, Britch SC (2006) Patterns of genetic variation in the adaptive radiation of New World crossbills (Aves: Loxia). *Molecular Ecology*, **15**, 1873–1887.
Parchman TL, Gompert Z, Mudge J *et al.* (2012) Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology*, **21**, 2991–3005.
Pastene LA, Goto M, Kanda N *et al.* (2007) Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata. Molecular Ecology*, **16**, 1481–1495.
Peterson BK, Weber JN, Kay EH *et al.* (2012) Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, **7**, e37135.
Petren K, Grant PR, Grant BR, Keller LF (2005) Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Molecular Ecology*, **14**, 2943–2957.
Pfenninger M, Staubach S, Albrecht C, Streit B, Schwenk K (2003) Ecological and morphological differentiation among cryptic evolutionary lineages in freshwater limpets of the nominal form-group *Ancylus fluviatilis* (O.F. Muller, 1774). *Molecular Ecology*, **12**, 2731–2745.
Potvin C, Bernatchez L (2001) Lacustrine spatial distribution of landlocked Atlantic salmon populations assessed across generations by multilocus individual assignment and mixed-stock analyses. *Molecular Ecology*, **10**, 2375–2388.
Price BW, Barker NP, Villet MH (2007) Patterns and processes underlying evolutionary significant units in the *Platypleura stridula* L. species complex (Hemiptera: Cicadidae) in the Cape Floristic Region, South Africa. *Molecular Ecology*, **16**, 2574–2588.
Prunier R, Holsinger KE (2010) Was it an explosion? Using population genetics to explore the dynamics of a recent radiation within Protea (Proteaceae L.). *Molecular Ecology*, **19**, 3968–3980.

Puebla O, Bermingham E, Guichard F (2008) Population genetic analyses of Hypoplectrus coral reef fishes provide evidence that local processes are operating during the early stages of marine adaptive radiations. *Molecular Ecology*, **17**, 1405–1415.
Puebla O, Bermingham E, McMillan WO (2014) Genomic atolls of differentiation in coral reef fishes (Hypoplectrus spp., Serranidae). *Molecular Ecology*, **23**, 5291–5303.
Puritz JB, Matz M V., Toonen RJ *et al.* (2014) Demystifying the RAD fad. *Molecular Ecology*, **23**, 5937–5942.
eymaekers JAM, Maes GE, Audenaert E, Volckaert FAM (2005) Detecting Holocene divergence in the anadromous-freshwater three-spined stickleback (*Gasterosteus aculeatus*) system. *Molecular Ecology*, **14**, 1001–1014.
Remington DL, Robichaux RH (2007) Influences of gene flow on adaptive speciation in the *Dubautia arborea-D. ciliolata* complex. *Molecular Ecology*, **16**, 4014–4027.
usch TBH, Wegner KM, Kalbe M (2001) Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographical proximity. *Molecular Ecology*, **10**, 2435–2445.
Rohland N, Reich D (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research*, **22**, 939–946.
Roy D, Paterson G, Hamilton PB, Heath DD, Haffner GD (2006) Resource-based adaptive divergence in the freshwater fish Telmatherina from Lake Matano, Indonesia. *Molecular Ecology*, **16**, 35–48.
Rundell RJ, Price TD (2009) Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution*, **24**, 394–399.
Rutschmann S, Matschiner M, Damerau M *et al.* (2011) Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. *Molecular Ecology*, **20**, 4707–4721.
Salamin N, Wüest RO, Lavergne S, Thuiller W, Pearman PB (2010) Assessing rapid evolution in a changing environment. *Trends in Ecology and Evolution*, **25**, 692–698.
Sanders KL, Rasmussen AR, Mumpuni J *et al.* (2013) Recent rapid speciation and ecomorph divergence in Indo-Australian sea snakes. *Molecular Ecology*, **22**, 2742–2759.
Schliewen U, Rassmann K, Markmann M *et al.* (2001) Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. *Molecular Ecology*, **10**, 1471–1488.
hlötterer C, Amos B, Tautz D (1991). Conservation of polymorphic simple sequence loci in cetacean species. *Nature*, **354**, 63–65.
Schlötterer C, Tobler R, Kofler R, Nolte V (2014) Sequencing pools of individuals — mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics*, **15**, 749–763.
hluter D (2000) The ecology of adaptive radiation. *Oxford University Press,* Oxford, pp 288.
Seehausen O (2004) Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, **19**, 198–207.
Seehausen O, Butlin RK, Keller I *et al.* (2014) Genomics and the origin of species. *Nat Rev Genet*, **15**, 176–192.
Shum P, Pampoulie C, Kristinsson K, Mariani S (2015) Three-dimensional post-glacial expansion and diversification of an exploited oceanic fish. *Molecular Ecology*, **24**, 3652–3667.
Simões M, Breitkreuz L, Alvarado M, *et al.* (2016) The evolving theory of evolutionary radiations. *Trends in Ecology and Evolution*, **31**, 27-34.
Smadja, C.M. & Butlin, R.K. (2011) A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology*, **20**, 5123–40.
Soltis PS, Soltis DE (2016) Ancient WGD events as drivers of key innovations in angiosperms. *Current Opinion in Plant Biology*, **30**, 159-165
St-Cyr J, Derome N, Bernatchez L (2008) The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.). *Molecular Ecology*, **17**, 1850–1870.

Steinkellner H, Lexer C, Turetschek E, Glössl J (1997) Conservation of (GA)(n) microsatellite loci between Quercus species. *Molecular Ecology*, **6**, 1189–1194.
Stervander M, Illera JC, Kvist L *et al.* (2015) Disentangling the complex evolutionary history of the Western Palearctic blue tits (*Cyanistes* spp.) - phylogenomic analyses suggest radiation by multiple colonization events and subsequent isolation. *Molecular Ecology*, **24**, 2477–2494.
Straub, S C, Parks, M, Weitemier, *et al.* (2012). Navigating the tip of the genomic iceberg: Next-generation sequencing for plant systematics. *American Journal of Botany*, **99**, 349-364.
Streicher JW, Devitt TJ, Goldberg CS *et al.* (2014) Diversification and asymmetrical gene flow across time and space: lineage sorting and hybridization in polytypic barking frogs. *Molecular Ecology*, **23**, 3273–3291.
Suchan T, Pitteloud C, Gerasimova NS *et al.* (2016) Hybridization capture using RAD probes (hyRAD), a new tool for performing genomic analyses on collection specimens. *PLoS ONE*, **11**.
Surget-Groba Y, Kay KM (2013) Restricted gene flow within and between rapidly diverging Neotropical plant species. *Molecular Ecology*, **22**, 4931–4942.
Takahashi T, Watanabe K, Munehara H, Rüber L, Hori M (2009) Evidence for divergent natural selection of a Lake Tanganyika cichlid inferred from repeated radiations in body size. *Molecular Ecology*, **18**, 3110–3119.
Taylor MS, Hellberg ME (2006) Comparative phylogeography in a genus of coral reef fishes: biogeographic and genetic concordance in the Caribbean. *Molecular Ecology*, **15**, 695–707.
The Heliconius Genome Consortium, Dasmahapatra KK, Walters JR *et al.* (2012) Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*, **487**, 94–98.
Thorpe RS, Jones AG, Malhorta A, Surget-Groba Y (2008) Adaptive radiation in Lesser Antillean lizards: molecular phylogenetics and species recognition in the Lesser Antillean dwarf gecko complex, *Sphaerodactylus fantasticus*. *Molecular Ecology*, **17**, 1489–1504.
Townsend AK, Rimmer CC, Latta SC, Lovette IJ (2007) Ancient differentiation in the single-island avian radiation of endemic Hispaniolan chat-tanagers (Aves: *Calyptophilus*). *Molecular Ecology*, **16**, 3634–3642.
Van Orsouw N J, Hogers R C, Janssen A *et al.* (2007) Complexity reduction of polymorphic sequences (CRoPS™): a novel approach for large-scale polymorphism discovery in complex genomes. *PloS ONE*, **2**, e1172.
Voje KL, Hemp C, Flagstad Ø, Saetre G-P, Stenseth NC (2008) Climatic change as an engine for speciation in flightless Orthoptera species inhabiting African mountains. *Molecular Ecology*, **18**, 93–108.
Vos P, Hogers R, Bleeker M *et al.* (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.
Wada S, Kameda Y, Chiba S (2013) Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. *Molecular Ecology*, **22**, 4801–4810.
Wagner CE, Keller I, Wittwer S *et al.* (2013) Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Molecular Ecology*, **22**, 787–798.
Warren B, Bermingham E, Prys-Jones RP, Thébaut C (2006) Immigration, species radiation and extinction in a highly diverse songbird lineage: white-eyes on Indian Ocean islands. *Molecular Ecology*, **15**, 3769–3786.
Wellborn GA, Broughton RE (2008) Diversification on an ecologically constrained adaptive landscape. *Molecular Ecology*, **17**, 2927–2936.
Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. *Trends in Ecology and Evolution*, **19**, 639–644.

Wilke T, Pfenninger M (2002) Separating historic events from recurrent processes in cryptic species: phylogeography of mud snails (Hydrobia spp.). *Molecular Ecology*, **11**, 1439–1451.
Wilson NG, Schrödl M, Halanych KM (2009) Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology*, **18**, 965–984.
Yuri T, Jernigan RW, Brumfield RT, Bhagabati NK, Braun MJ (2009) The effect of marker choice on estimated levels of introgression across an avian (Pipridae: Manacus) hybrid zone. *Molecular Ecology*, **18**, 4888–4903.
Zhang D-X, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular ecology*, **12**, 563–584.
Zidana H, Turner GF, van Oosterhout C, Hänfling B (2009) Elevated mtDNA diversity in introduced populations of *Cynotilapia afra* (Günther 1894) in Lake Malawi National Park is evidence for multiple source populations and hybridization. *Molecular Ecology*, **18**, 4380–4389.
Zohren J, Wang N, Kardailsky I, Borrell JS, Joecker A, Nichols RA, Buggs RJ (2016) Unidirectional diploid-tetraploid introgression among British birch trees with shifting ranges shown by restriction site-associated markers. *Molecular Ecology*, **25**, 2413-2426

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Fig. 1. Summary of the reviewed articles by publication year, separated into studies situated within and outside of recognized world biodiversity hotspots. No studies of radiations outside of biodiversity hotspots were identified with our search terms before 2001.

Fig. 2. Summary of the reviewed articles by taxonomic group, broken down by the number of *a priori* recognized species included in each study.

Fig. 3. Prior species numbers (*x* axis; *a priori* species designations based on traditional taxonomy) *versus* posterior species numbers (*y* axis; species designations after applying molecular markers) colour-labelled by taxonomic group. Circle sizes are proportional to the mbers of studies represented by each data point. For generalized linear mixed effect models (GLM's) explaining the relationship between prior and posterior species numbers see text and **Table S2**. Two studies with both prior and posterior species numbers of 33 and respectively, were included in GLM's but are not depicted in the graph for better visibility of the remaining data points. The diagonal allows inspecting departures of the data from a perfect linear relationship.

Fig. 4. Major concepts, scales, and approaches currently relevant to molecular ecology studies of species radiations, including concepts and approaches expected to advance research on radiations in the coming years. **A**, Hypothetical phylogeny of radiating taxa, with orange and green circles representing ecologically important traits (e.g. traits facilitating the invasion of a new adaptive zone). The orange trait is associated with rapid diversification, i.e. radiation. Orange and green taxa differ in their altitudinal ranges, with green taxa found at er altitudes and orange taxa at higher altitudes, as may be the case for plant taxa with different pollination syndromes. Latitudinal delimitation of orange and green clades is only for se of visualization. The inset under the magnifying glass shows the population geneticist's angle of studying radiations, e.g. using tree- or network-based approaches. Here, yellow, orange, and red circles indicate locally adapted populations or ecotypes with differences in adaptive traits, exemplified by elevational adaptation in mountains. The transition between the micro- and macro-evolutionary scales (or between phylogenetics and population genetics, respectively) is in fact more continuous than depicted in the graph. **B**, Different molecular and genomic approaches currently in use for studying radiations, including their

practicability across different taxonomic ranks and levels of divergence. Whole genome sequencing (WGS) is potentially applicable across a wider taxonomic range than depicted here, when considering study designs that specifically target conserved coding regions (thus discarding the bulk of more divergent sequence reads obtained from intergenic space), and so-called ´genome skimming´ approaches targeting primarily organellar and ribosomal DNA.

Supporting Information accompanying this paper:

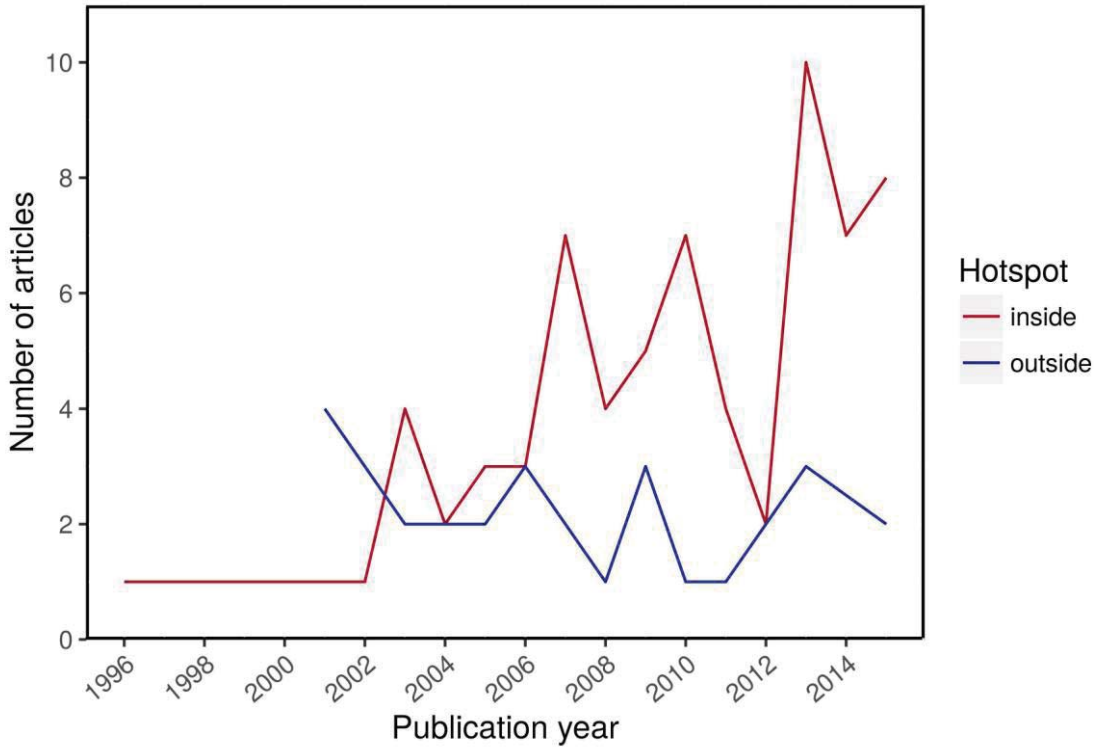
Table S1. Essential biological and technical features recovered from 93 studies on species radiations published in MOLECULAR ECOLOGY over a 20-year period. Full citations of all articles are provided in the reference section of this paper.

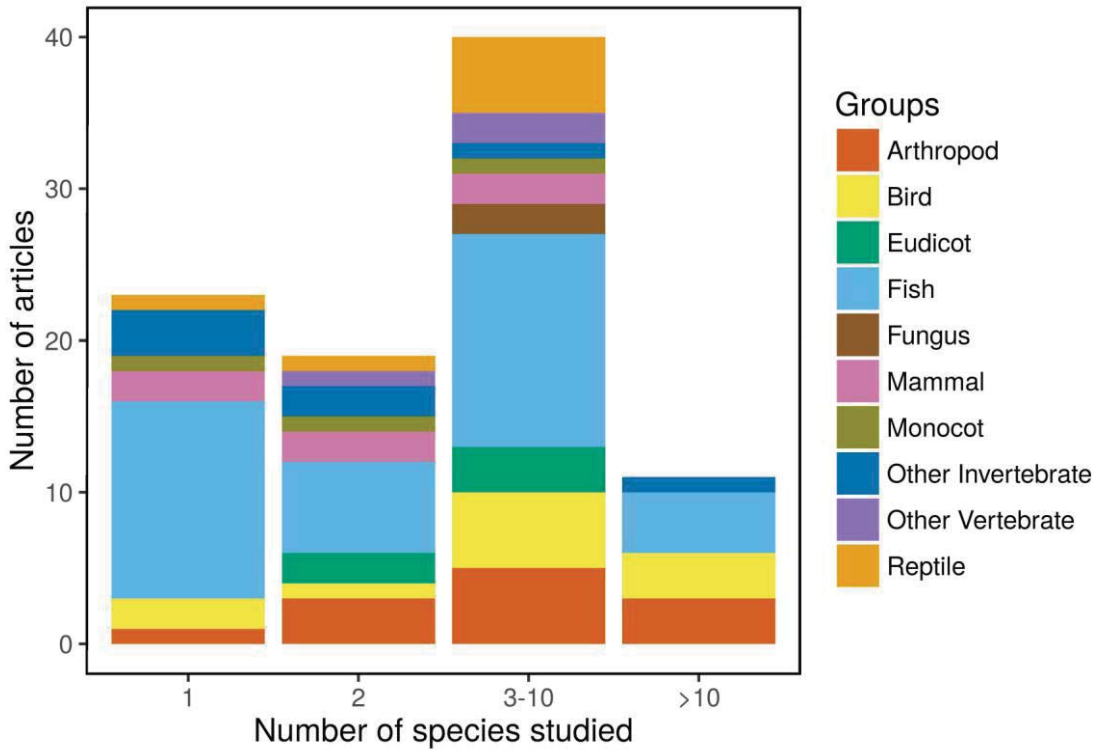
Table S2: Performance of different predictors and models to explain the difference between prior and posterior numbers of species in the articles reviewed for this Opinion paper. Abbreviations: prior_posterior, difference between prior and posterior number of species; genome, type of genome sampled, i.e. either including nuclear genomes or organellar genomes only; year, publication year of study, no_pop, number of populations sampled; nb_ind, total number of individuals sampled for each study; ind_per_pop, number of individuals per population. For graphical representation of prior *versus* posterior species numbers see **Fig. 3** of main paper.

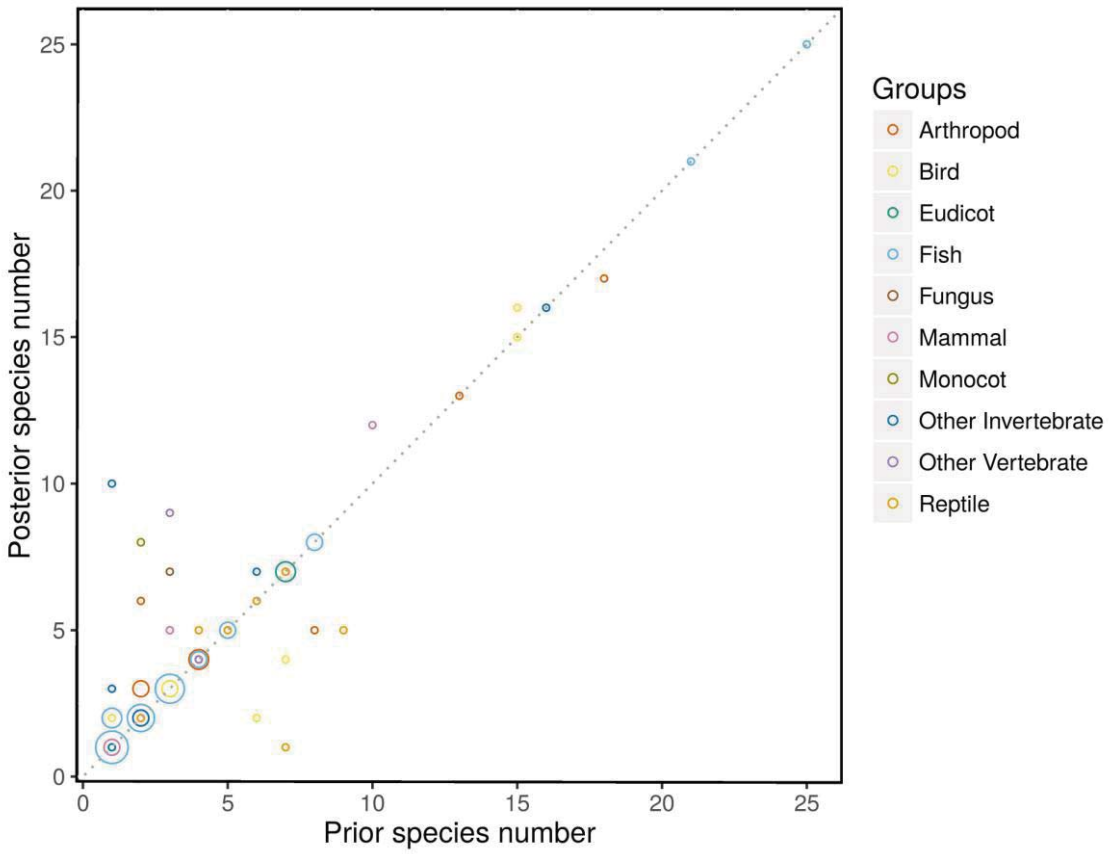
Table 1. Summary of molecular ecology studies of species radiations reviewed for this Opinion paper.

Category[1]	No. of items
Total no. of reviewed studies	93
Within hotspots	69
Outside hotspots	24
By biogeographic region	
Mediterranean	9
Polar	4
Temperate	20
Tropical	49
Tropical-temperate	5
Widespread / other	6
By molecular method[2]	
AFLP[3]	6
Microsatellites	28
Nuclear sequencing[4]	26
Organellar sequencing[4]	60
NGS genotyping[5]	13
Genomic / other[6]	6

[1]For study counts in different organismal groups see **Fig. 1**, for complete review database analyzed see **Table S1**. [2]Numbers exceed the total no. of studies, because many studies employed more than one molecular method. [3]Amplified Fragment Length Polymorphism.[4]Mainly Sanger sequencing of PCR amplicons. [5]Studies employing GBS or RAD-seq. [6]Includes RNA-seq and microarray-based gene expression studies.







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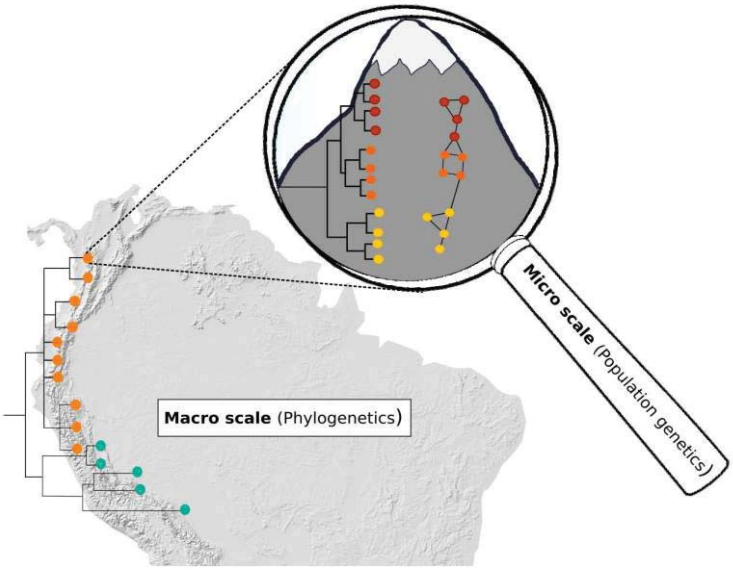

B.